# THE RELATION OF INFLAMMATION TO THE MOLECULAR STRUCTURE OF CARBON COMPOUNDS SOLUBLE IN THE FLUIDS OF THE BODY

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In a foregoing publication (1) evidence was assembled to show that the activity of an inflammatory reaction following the injection of an injurious substance into the peritoneal cavity could be measured by the quantity of fluid within the cavity after intervals following injection, by the number of leucocytes that entered the cavity, and by the concentration of plasma protein in the peritoneal fluid. The activity of reactions caused by solutions of electrolytes was found to vary widely with the chemical constitution of those that were tested. It was in accord with molecular weight, ion dissociation, and valence. The latter was the dominant factor varying with multiples of one, two, or three or more, as determined by the acid or basic ions of salts. These relations were in agreement with the colligative properties of solutions of electrolytes, namely, with their osmotic pressure, freezing point depression, vapor pressure, and electric conductivity. As it is well known the relation between these properties is such that from any one of them the others can be derived by calculation (Taylor, reference 2).

The present experiments were undertaken to measure the activity of inflammatory reactions caused by non-electrolytes and include compounds which are of most interest from the standpoint of human pathology, that is, carbon compounds. These are in part soluble in the water of the body, as saccharides, some alcohols, many proteins, and related compounds. Some other inflammatory irritants are insoluble in water but soluble in lipoids. These include oils, halogen derivatives of methane, and compounds of the benzene series, cited as examples.

## Method

Fluid present in one of the large serous cavities of the body after injection of an injurious solution can be measured with considerable accuracy (3). White rats weighing usually from 150 to 200 gm have received no food overnight preceding the experiment. The quantity of solution injected has been in most instances 2 cc per 100 gm of body weight of each animal because this quantity was found to be well adjusted to the large capacity of the serous cavities of the white rat (designated as 2 cc/100 gm).

Dilute solutions of irritant substances may be more or less rapidly absorbed but when stronger solutions are injected the fluid content of the cavity increases in excess of that which is injected. When solutions in graded concentration were used the per cent of decrease with

absorption of fluid (— per cent) or increase with exudation (+ per cent) was measured directly. These changes varied with the activity of the inflammatory reaction and were recorded after 1 hour at an early stage of inflammation or after 3 hours when it was well established. They were determined by the permeability of the complex membrane which intervenes between the blood stream and the cavity. It is noteworthy that this membrane is formed by endothelial cells of the blood vessels, fixed tissue, and mesothelium of the serous cavity.

The number of leucocytes that have entered the fluid were counted in 1 mm$^3$ and the total number was recorded after division for convenience by 10$^5$. The protein content of the fluid in the cavity was measured by its turbidity after precipitation by 5 per cent trichloracetic acid made up in a solution 0.25 per cent saturated by ammonium sulfate (Kunitz and Northrop, reference 4).

The experiments have been made with the able assistance of Miss Marika Tershakovec.

## RESULTS

*Inflammation Caused by Saccharides.*—The activity of inflammatory reactions caused by the saccharides that were tested, namely, 2 polysaccharides, 4 hexoses, and 3 pentoses, was measured after 1 and after 3 hours following injection into the peritoneal cavity. Absorption of fluid indicated by decreased quantity in the cavity or exudation by increase above that which was injected occurred with some exceptions in accord with the decreasing molecular weight of these substances (Table I, Part I after 1 hour, and Part II after 3 hours).

Each substance was tested in low molar concentration and later tests were repeated until the molar level of increased peritoneal fluid was found. Stachyose and raffinose in 0.1 M solutions did not prevent absorption during 1 hour and 0.15 M solutions caused exudation (+27 per cent and +37 per cent respectively); solutions, 0.2 M, after 3 hours caused increase above that injected (+48 per cent and +46 per cent). The hexose, maltose, caused exudation after 1 hour at 0.15 M, but after 3 hours only at 0.4 M. Sucrose and sorbose maintained the level of injected fluid after 1 hour when in concentration 0.2 M and with the exception of glucose in 0.3 M after 3 hours; glucose failed to cause exudation after 3 hours at this level. The pentoses, ribose and xylose, 0.2 M, caused exudation during 1 hour; arabinose 0.3 M had similar effect. After 3 hours these pentoses caused exudation in solutions 0.3, 0.4, and 0.5 M respectively.

The permeability of the membrane between circulating blood and peritoneal cavity as measured by fluid exudation increased in accord with increase of the molecular weight of the saccharides that were tested. The quantity of fluid within the cavity was determined with accuracy but was doubtless subject to variations determined by the individual conditions incident to the use of any experimental animal.

Leucocytes entered the peritoneal cavity in large number following injection of solutions of saccharides and increased in the period from 1 to 3 hours after injection. These relations are shown by Table II in which is recorded for each saccharide the number brought into the peritoneal cavity by the weakest molar

solution which caused fluid increase in excess of the quantity injected, that is, caused exudation.

The protein concentration of peritoneal fluid after 1 hour was less than one-tenth that of the plasma of the blood (1) and after 3 hours slightly greater than one-tenth (Table II).

TABLE I

*Fluid Movement Following Injection of Molar Solutions of Saccharides into the Peritoneal Cavity*

The table records the per cent increase (+ per cent) or decrease (−%) of peritoneal fluid following injection of solutions 2 cc per 100 gm of body weight of each experimental animal (2 cc/100 gm).

| Saccharides | Mol. wt. | 0.1 M | 0.15 M | 0.2 M | 0.3 M | 0.4 M | 0.5 M |
|---|---|---|---|---|---|---|---|
| *Part I: after 1 hr.* | | | | | | | |
| | | % | % | % | % | % | % |
| Stachyose | 666.6 | −2 | +27 | | | | |
| Raffinose | 594.52 | −6 | +37 | | | | |
| Maltose | 360.31 | −30 | +37 | +36 | +77 | | |
| Sucrose | 348.36 | −22 | −4 | +47 | +97 | | |
| Glucose | 180.16 | | −19 | +18 | +28 | | |
| Sorbose | 180.16 | | −29 | +25 | +58 | | |
| Ribose | 150.13 | | −24 | +20 | +34 | | |
| Xylose | 150.13 | | −39 | +18 | +43 | | +114 |
| Arabinose | 150.13 | | −16 | −1 | +40 | | +118 |
| *Part II: after 3 hrs.* | | | | | | | |
| Stachyose | | | | −4 | +48 | | |
| Raffinose | | | | −20 | +46 | | |
| Maltose ⎫ | | | | −33(2) | −27 | +50 | |
| Sucrose ⎪ Hexose | | | −91 | +32 | +73 | +95 | |
| Glucose ⎪ | | | | −66 | −34 | −7 | |
| Sorbose ⎭ | | | | −45 | −14 | +14 | |
| Ribose ⎫ | | | | −68 | +67 | | |
| Xylose ⎬ Pentose | | | −51 | −39 | −18 | +6 | +97 |
| Arabinose ⎭ | | | −86 | −75 | −21 | −2 | +58 |

*Inflammation Caused by Alcohols.*—A series of normal alcohols with increasing length of the carbon chain and increasing molecular weight offered opportunity to compare the occurrence of these properties with ability to promote inflammatory reactions. The rapidity with which these alcohols were absorbed and their toxicity when injected into the peritoneal cavity were evident. Ethyl alcohol injected into the peritoneal cavity in concentration from 0.15 to 4.0 M/100 gm was in great part absorbed during 1 hour. A solution 0.3 M gm caused coma.

Amyl alcohol was fatally toxic when injected 0.5 M/100 gm and butyl 1.0 M/100 gm.

More information seemed to be obtainable if the alcohols were injected with sodium chloride 0.3 M as in foregoing experiments (1) undertaken to measure the activity of inflammatory reactions.

A series of normal alcohols, methyls, ethyl, propyl, butyl, and amyl alcohol, with increasing molecular weight were tested, and the cubic centimeter of a 1 per cent solution of each needed to cause exudation of fluid during 1 and

TABLE II

*Leucocytes and Plasma Protein in Peritoneal Fluid After Injection of the Least Molar Solution of Saccharides that Caused Exudation of Fluid*

Accumulation of leucocytes (divided by $10^5$) and exudation of plasma protein (units of turbidity as described in text) after peritoneal injection of saccharides (as for Table I).

| Saccharides | Leucocytes ÷ $10^5$ | | Protein turbidity units | |
|---|---|---|---|---|
| | after 1 hr. | after 3 hrs. | after 1 hr. | after 3 hrs. |
| Stachyose | 241 | 246 | 79 | 89 |
| Raffinose | 175 | 258 | 87 | 128 |
| Maltose | 180 | 496 | 75 | 180 |
| Sucrose | 262 | 399 | 78 | 75 |
| Glucose | 220 | 477 | 97 | 226 |
| Sorbose | 252 | 267 | 85 | 113 |
| Ribose | 180 | 434 | 135 | 290 |
| Xylose | 200 | 294 | 104 | 187 |
| Arabinose | 200 | 352 | 86 | — |
| Average | 212 | 358 | 92 | 161 |

during 3 hours was determined. The molar concentration of these solutions is shown by Table III. The molar concentration of each alcohol required to cause exudation of fluid into the peritoneal cavity in excess of that which was injected was as follows:

| | After 1 hr. | After 3 hrs. |
|---|---|---|
| | M | M |
| Amyl alcohol | 0.01 | Fatal |
| Butyl | 0.01 | 0.1 |
| Propyl | 0.01 | 1.0 |
| Ethyl | 0.15 | 1.5 |
| Methyl | 0.15 | 2.5 |

The concentration of these alcohols needed to cause exudation of fluid increased in the series from amyl to methyl in accord with their molecular weight

This relation was more evident after 3 hours when inflammation was well established. It is noteworthy that the boiling point of these alcohols is in accord with increasing molecular weight (Table III).

In all instances leucocytes entered the cavity during 1 hour and continued to increase during 3 hours. The concentration of protein after 3 hours with the 5 alcohols was approximately half that of the blood plasma. With increasing injury to the peritoneum red blood corpuscles passed from the blood vessels into the peritoneal exudate and gave it a red color. Severe peritoneal inflamma-

### TABLE III

*Fluid Movement Following Intraperitoneal Injection of Alcohols and Sodium Chloride 0.3 M 2 cc/100 gm*

The table records the number of cubic centimeters of alcohol which when added to sodium chloride 0.3 M 2 cc/100 gm were required after 1 (Part I) and 3 (Part II) hours to produce fluid in excess of that injected. Retraction of the omentum (R) and fatal toxicity (D) were caused by the quantities indicated.

| Molar concentration | | 0.05 % | 0.1 % | 0.2 % | 0.5 % | 1.0 % | 1.5 % | 2.0 % | 2.5 % | 3.0 % | 3.5 % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Part. I: after 1 hr.* | | | | | | | | | | | |
| | Mol. wt. | | | | | | | | | | |
| Amyl | 88.13 | −26 | +14 | +18 | D | | | | | | |
| Butyl | 74.12 | | +2 | +15 | +36R | D | | | | | |
| Propyl | 60.09 | | +15 | +9 | +23R | +48 | | | | | |
| Ethyl | 46.07 | | | | −4 | +0 | +34 | +45R | +54R | | |
| Methyl | 32.04 | | | | | −14 | +13 | +3 | +7 | +32 | +49R |
| *Part II: after 3 hrs.* | | | | | | | | | | | |
| | Boiling point | | | | | | | | | | |
| Amyl | 138–139°C | −82 | −51 | −24 | D | | | | | | |
| Butyl | 117–118°C | | | −8 | +39R | | | | | | |
| Propyl | 97–98°C | | | −25 | −13 | +54R | | | | | |
| Ethyl | 78.5°C | | | | | −42 | +8 | +49 | +101R | | |
| Methyl | 64.7°C | | | | | | | −53 | +20 | +48 | +91R |

tion was accompanied by retraction (R) of the omentum (Table III, 7) along the greater curvature of the stomach. It was caused by deposition of leucocytes and of fibrin upon the surface of the membrane and is a conspicuous feature of active inflammatory reactions. Retraction of the omentum was caused by 0.5 M butyl alcohol, by 0.5 M propyl alcohol, by 2 M ethyl alcohol, and by 3.5 M methyl alcohol.

*Changes Following the Injection of Proteins into the Peritoneal Cavity.*—Blood serum was obtained from blood removed during life from the heart of rats and was injected into the peritoneal cavity 2 cc/100 gm. Water movement was almost identical with that which followed similar injection of sodium chloride 0.15 M (Table IV). There was with the blood serum greater migration of leucocytes between 1 and 3 hours and as might be expected protein in the peritoneal fluid was equal to that in blood plasma.

Bovine alpha globulin (fraction IV-I) prepared by the method of Cohn (5) and supplied by Nutritional Biochemicals Corporation, Cleveland, was injected into the peritoneal cavity in a concentration approximately equal to that of the total protein in blood serum, 6.4 per cent, in order to compare the resulting changes with those caused by blood serum and by sodium chloride 0.15 M (Table IV). Absorption of fluid was retarded but migration of leucocytes and protein content of the peritoneal fluid was only slightly less than that with blood serum.

Experiments were made to measure the activity of inflammatory reactions caused by proteins with low molecular weight. A group of such proteins used by Craig and his associates (6) for study of dialysis through selected cellophane

TABLE IV

*Movement of Fluid, Leucocytes and Protein in the Peritoneal Cavity after Injection of Blood Serum, and of Globulin, Compared with Corresponding Changes after Injection of Sodium Chloride 0.15 M*

|  | Fluid after | | Leucocytes after | | Protein after | |
|---|---|---|---|---|---|---|
|  | 1 hr. | 3 hrs. | 1 hr. | 3 hrs. | 1 hr. | 3 hrs. |
| Blood serum of rat | −17 | −75 | 155 | 361 | 480 | 460 |
| Bovine alpha globulin | −30 | −39 | 74 | 295 | 463 | 453 |
| Sodium chloride 0.15 M | −57 | −74 | 154 | 285 | 146 | 274 |

membranes were suitable for the purpose. Their molecular weight varying from that of cytochrome C, 12,000, to that of ovalbumin, 45,000. The time during which 50 per cent of the solution passed through the membrane increased in accord with the increasing molecular weight of the protein from 60 minutes to 80 hours.

Even when molecular weights are as low as those just cited it is not possible to measure the activity of inflammatory reactions in terms of the molar concentrations needed to produce a definable change. It seemed desirable to measure water movement in the peritoneal cavity when a uniform quantity of a solution was injected and for this purpose in each instance a one per cent solution was used in quantity of 2 cc per 100 gm of body weight. Fluid that entered passed through the complex membrane which separates the blood stream from the cavity. The substances that were tested and their molecular weights are listed in Table V.

After a half hour with two noteworthy exceptions from 59 to 76 per cent of each of the injected solutions had left the cavity (Fig. 1). After 1 and after 3 hours there was somewhat wider variation but the graph shows that fluid movement occurred within a restricted zone so that after 1 hour the quantity

## TABLE V

*The Movement of Fluids in the Peritoneal Cavity after Injection of 1 per cent Solutions of Proteins of Low Molecular Weight in Quantity 2 cc/100 gm*

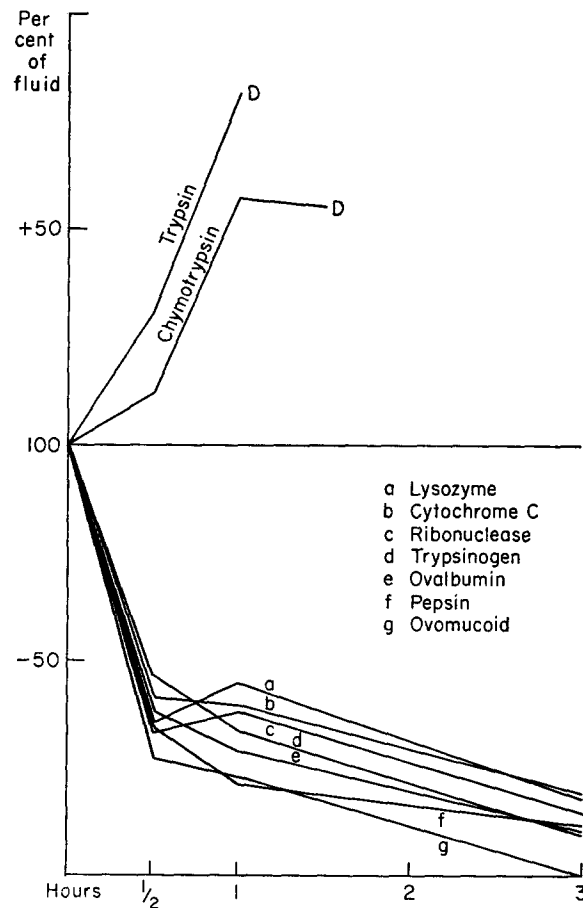|  | Mol. wt. | ½ hr. | 1 hr. | 2 hrs. | 3 hrs. |
|---|---|---|---|---|---|
| Cytochrome C | 12,000 | −67 | −62 |  | −83 |
| Ribonuclease | 13,000 | −66 | −65 |  | −95 |
| Lysozyme | 14,000 | −65 | −69 |  | −82 |
| Trypsin | 20,000 | +23 | +D | +D |  |
| Trypsinogen | 20,000 | −59 | −63 |  | −86 |
| Chymotrypsin | 25,000 | +25 | +65 | +60D |  |
| Ovomucoid | 28,000 | −69 | −72 |  | −100 |
| Pepsin | 35,000 | −76 | −74 |  | −82 |
| Ovalbumin | 45,000 | −64 | −69 |  | −90 |



FIG. 1. Movement of fluid within the peritoneal cavity after injection of 1 per cent solutions of proteins of low molecular weight in quantity 2 cc for 100 gm of body weight of experimental animals (see Table V).

493

of fluid absorbed was from 61 to 79 per cent; after 3 hours from 82 to 100
per cent.

After injection of cytochrome C, trypsinogen, and ovalbumen, leucocytes
increased continuously and with the progress of inflammation became abundant
after 3 hours (Table VI). Pepsin and lysozyme caused less migration of leu-
cocytes. In all instances protein increased in the fluid within the peritoneal
cavity above the level of that which was injected.

TABLE VI

*Leucocytes and Protein in Exudates Caused by the Injection of Protein 2 cc/100 gm
into the Peritoneal Cavity*

|  | Leucocytes | | | Protein | | |
|---|---|---|---|---|---|---|
|  | ½ hr. | 1 hr. | 3 hrs. | ½ hr. | 1 hr. | 3 hrs. |
| Cytochrome C | 13 | 77 | 275 | 450 | 405 | — |
| Ribonuclease | 33 | 59 | — | — | 320 |  |
| Lysozyme | 31 | 24 | 52 | 40 | 20 | 396 |
| Trypsin | 296 | Fatal | Fatal | 300 | Fatal | Fatal |
| Trypsinogen | 8 | 12 | 120 | 132 | 174 | — |
| Chymotrypsin | 272 | 298 | D | 300 | 325 | D |
| Ovomucoid | 46 | 60 | — | 300 | 330 | — |
| Pepsin | 79 | 56 | 41 | — | — | — |
| Ovalbumin | 44 | 73 | 142 | 226 | 199 |  |

TABLE VII

*Leucocytes and Protein in Exudates Caused by the Injection of 1 per cent Solutions of
Trypsin and of Trypsinogen into the Peritoneal Cavity in Quantity 1 cc/100 gm*

|  | Fluid | | | Leucocytes | | | Protein | | |
|---|---|---|---|---|---|---|---|---|---|
|  | ½ hr. | 1 hr. | 3 hrs. | ½ hr. | 1 hr. | 3 hrs. | ½ hr. | 1 hr. | 3 hrs. |
| Trypsin 1 cc/100 | +38 | +82 | +283 | 215 | 284 | 423 | 430 | 335 | 310 |
| Trypsinogen 1 cc/100 | −73 | −77 | −95 | 10 | 13 | 145 | — | — | — |

Trypsin and chymotrypsin proved to be active inflammatory irritants and
their activity agreed with their ability to cause disintegration of proteins.
Pepsin under the conditions of these tests and in the absence of acidity did
not cause similar changes.

After trypsin had been found to be fatally toxic when given 2 cc/100 gm the
quantity was reduced to 1 cc/100 gm and the result compared with that of
trypsinogen when injected in the same quantity (Table VII). Trypsin caused an
exudation of fluid greatly in excess of the fluid injected and there was migration
of leucocytes in large number. Trypsinogen was a mild irritant permitting ab-
sorption of fluid and producing moderate accumulation of leucocytes.

Trypsin in 1 per cent solution 2 cc/100 gm caused widespread dilatation of blood vessels of the peritoneum of the small intestine, mesentery, and parietal peritoneum. Spots of diffuse injection of the omentum and of fat projecting from the pelvis into the abdominal cavity were seen. The exuded fluid was red and contained red blood corpuscles in considerable quantity. There was some hemolysis evident when peritoneal fluid was centrifuged. Similar changes were found in animals that received 1 per cent solution 1 cc/100 gm (Table VII).

Inflammatory changes and toxicity of chymotrypsin were like those of trypsin. In 1 per cent solution 2 cc/100 gm it was fatally toxic after approximately 2 hours. During $\frac{1}{2}$ hour peritoneal fluid increased 25 per cent and during 1 hour, 65 per cent. Leucocytes migrated in large number, $298 \times 10^5$, and protein was abundant in the peritoneal fluid. Dilatation of peritoneal blood vessels and diapedesis of red blood corpuscles were even more conspicuous than with trypsin, and hemolysis occurred. Diffuse injection of the peritoneal surfaces occurred apparently with some hemorrhaging in places in the omentum and its edge was retracted along the greater curvature of the stomach near the pylorus. Fat projecting from the pelvis into the abdominal cavity was diffusely red.

The significance of the active inflammatory reactions caused by trypsin and chymotrypsin will be discussed later.

*Inflammation Caused by Injection of "Peptone" into the Peritoneal Cavity.*—In earlier experiments the effect of a nutrient medium containing "peptone" and adapted to the growth of bacteria was injected into the peritoneal or pleural cavity in order to compare the resulting changes with those caused by cultures of *Staphylococcus aureus* or of *Escherichia coli* grown in the same medium. It produced active inflammatory reactions less severe than those that followed injection of the bacterial cultures.

Experiments were made to determine if peptone, long regarded as favorable for. bacterial growth (as "peptone, U.S.P." of Matheson Coleman and Bell, East Rutherford, New Jersey), acted as an inflammatory irritant when injected into the peritoneal cavity. This peptone was added to sodium chloride 0.3 M 2 cc/100 gm as in foregoing experiments. Intraperitoneal injection of 0.002 cc of peptone in sodium chloride was followed by fluid absorption with loss of 40 per cent of fluid within 1 hour; 0.005 cc of peptone caused increase of fluid 16 per cent (Table VIII). When 0.02 cc was similarly injected fluid increase after 1 hour was 29 per cent; when 0.05 cc was injected there was after 3 hours an increase of 43 per cent. Leucocytes accumulated in great number (409 to 558 $\times$ 10$^5$) 3 hours after injection of sodium chloride 0.3 M containing 0.02 to 0.05 cc of peptone. Exuded protein in the fluid was more than one-tenth that of blood plasma.

*Inflammatory Reactions Following the Injection of Polypeptides in to Serous Cavities.*—Inflammatory changes caused by intraperitoneal injection of several synthetic polypeptides kindly supplied by Sandoz Pharmaceuticals, Hanover,

TABLE VIII

*Fluid Movement. Leucocytes and Protein in the Peritoneal Cavity after Injection of Peptone with Sodium Chloride 0.3 M*

| Sodium chloride | Peptone | After 1 hr. | | | After 3 hrs. | | |
|---|---|---|---|---|---|---|---|
| | | Fluid | Leuco-cytes ÷ 10⁵ | Protein units | Fluid | Leuco-cytes ÷ 10⁵ | Protein units |
| | | *per cent* | | | *per cent* | | |
| 2 cc/100 gm | 0.002 | −40 | | | | | |
| | 0.005 | +16 | 358 | | | | |
| | 0.01 | +36 | 278 | 83 | | | |
| | 0.02 | +29 | 306 | 82 | −31 | 558 | 208 |
| | 0.03 | | | | −3 | 409 | 152 |
| | 0.05 | | | | +43 | 517 | 143 |

TABLE IX

*Changes in Movement of Peritoneal Fluid, Leucocytes and Protein after Injection of Polypeptides*

| | | After 1 hr. | | | | | | After 3 Hrs. | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Fluid | Leuco-cytes ÷ 10⁵ | Pro-teins | Fluid | Leuco-cytes ÷ 10⁵ | Pro-teins | Fluid | Leuco-cytes ÷ 10⁵ | Pro-teins |
| | Mg in 1 cc | % | | *u.* | % | | *u.* | % | | *u.* |
| Bradykinin | 0.1 | −2 | 147 | 290 | | | | | | |
| Kallidin | 0.07 | +15 | 148 | 214 | +26 | 177 | 214 | −71 | 105 | — |
| Eldoisin | 0.1 | ±0 | 203 | 175 | +19 | 224 | 166 | | | |
| | Internat. blood pressure units in 1 cc | | 0.5 cc | | | 1 cc | | | | |
| Lysin 8-vaso-pressin | 270 | −17 | 192 | 185 | −28 | 189 | 186 | −87 | 92 | — |
| PLV⁵ vasopressin | 55 | −17 | 163 | 165 | −15 | 170 | 175 | | | |
| Oxytocin | 5 | | | | −21 | 116 | 175 | | | |

New Jersey, will be described. Of these polypeptides, three, namely, brady-kinin, kallidin, and eldoisin injected into the venous system had been found to cause dilatation of blood vessels and decrease of blood pressure. The quantity of each polypeptide in 1 cc of fluid is shown in Table IX.

Injection of 0.5 mg bradykinin and of eldoisin 0.5 mg caused during 1 hour water movement approximately the same as that with sodium chloride alone; after injection of kallidin 0.35 mg fluid entered the peritoneal cavity in larger quantity (Table IX). After injection of 1.0 cc of kallidin and of eldoisin there was fluid exudation in excess of that caused by sodium chloride 0.3 M.

A hormone from the posterior lobe of the pituitary was found by Du Vigneaud

(7) to occur as a chain of amino acids with arginine in position 8 of the chain. It inhibited diuresis, contracted smooth muscle of the small intestine and caused vasoconstriction with hypertension. Du Vigneaud and his coworkers identified a second vasopressin differing from the first by the presence of lysine in place of arginine in position 8.

The polypeptide designated as 2-phenylalanine-8-lysine (PLV-2) was described by Boissonnas and his coworkers (8). It differs from 8-lysine vasopressin by the presence in position 2 of phenylalanine in place of tyrosin. It contracts smooth muscle of small intestine and causes vasoconstriction and hypertension.

Synthetic oxytoxin has the structure of the hormone obtained from the posterior pituitary lobe. It stimulates contraction of the uterus and has been much used in obstetric practice.

## RECAPITULATION AND DISCUSSION

Foregoing studies have assembled evidence to show that changes which follow injury and are insufficient to cause local death of the injured tissue are followed by the mobilization of fluid, cells, and exuded proteins at the site of injury. When inflammation pursues a favorable course, peritoneal fluid and its contents are absorbed and inflammation ends with resolution, restoring the affected part to normal. The serous cavities of the body localize the process so that fluid can be measured, cells counted, and exuded protein determined. These measurements have proven accurate enough to reveal the progress of the reactions which follow injury, even though they may be altered by individual variations of the experimental animals, caused perhaps by unrecognized disease. In some instances the course of inflammation may be changed when the injurious agent has specific action on some organ outside of the field of local injury, that is, in the present experiments the peritoneum. Inflammation may be abruptly ended by fatal toxicity. Increase in the activity of inflammatory reactions in accord with increase of the quantity of the injected material has given evidence that the experiments are trustworthy (see Fig. 2).

The injection of a volume of fluid constant in relation to the body weight of the experimental animal makes it possible to use solutions graded in relation to their increasing molar concentration as an index of the activity of solutions of different compounds. Changes during 1 hour represent an early stage of inflammation and those after 3 hours a time when it is well established.

The mildest form of inflammation is promptly followed by absorption of fluid, and there is scant accumulation of leucocytic and of exuded plasma. With active inflammation blood vessels of the peritoneal surfaces are conspicuously dilated and red blood corpuscles appear in the exudate; hemolysis may follow. A more severe change is indicated by such injury to blood vessels that hemorrhage occurs in the wall of the small intestine, especially in its distal part and at times in scattered loops. The wall becomes swollen, rigid, and hemorrhagic
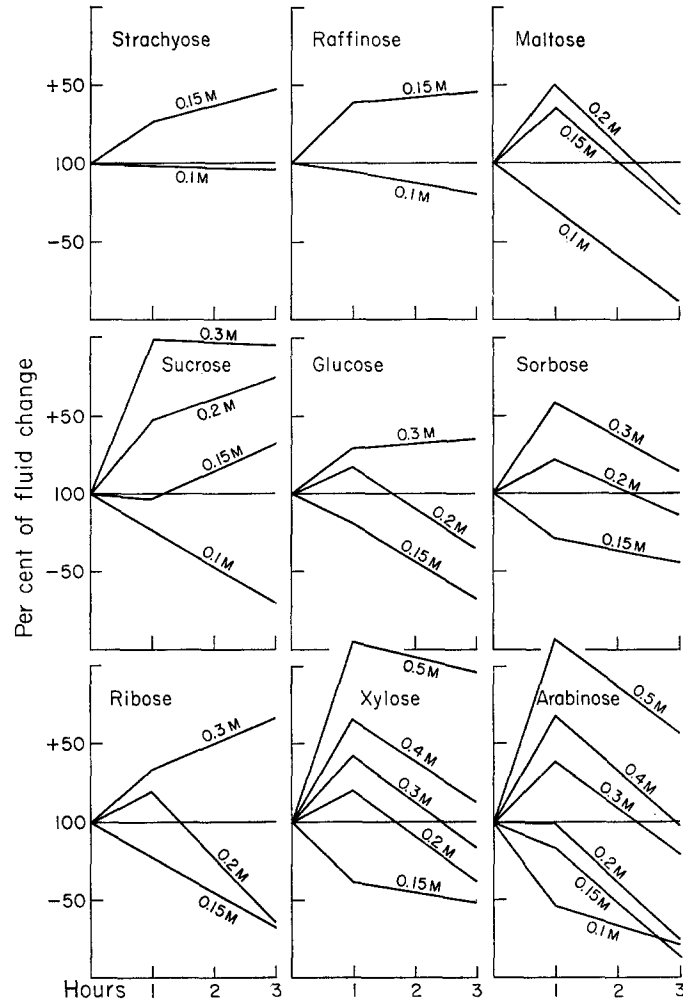
FIG. 2. Movement of fluid within the peritoneal cavity following injection of molar solutions of saccharides in quantity 2 cc for 100 gm of body weight of experimental animals (see Table I).

in appearance. With these changes blood enters the lumen of the intestine. A significant feature of very active inflammatory reactions within the peritoneal cavity is retraction of the omentum along the greater curvature of the stomach resulting from the deposition of leucocytes and fibrin upon the surface of the membrane. It is noteworthy that leucocytes deposited upon the peritoneal

surfaces diminish the number that can be counted in measured quantities of the peritoneal fluid.

The level of molar concentration necessary to produce exudation of fluid was most with polysaccharides, less with hexoses, and least with pentoses, diminishing in accord with the molecular weight of the substances that were tested. These relations were more conspicuous after 3 hours than after 1 hour. Migration of leucocytes and exudation of plasma protein occurred with similar relation to molecular weight. It is noteworthy that the osmotic pressure maintained by solutions of these compounds is in accord with their molecular weight (West, reference 9).

Alcohols in series with increasing molecular weight have caused inflammatory reactions with activity increasing in accord with these molecular weights The molar concentration of the normal alcohols, methyl, ethyl, propyl, butyl, and amyl needed to cause inflammation of corresponding activity increased with the length of their carbon chains with their molecular weight, and with their boiling points.

Blood serum of the rat or globulin in the concentration in which it occurs in blood serum injected into the peritoneal cavity was followed by changes which were almost the same as those caused by injection of sodium chloride 0.15 M. Injection of proteins adjusted to their molar concentration was not possible even when their molecular weight was as low as that of cytochrome C (12,000) or of ovalbumin (45,000). Injection of a measured quantity of a 1 per cent solution, that is 2 cc/100 gm, was followed by rapid and almost uniform absorption except in two instances; trypsin and chymotrypsin caused active inflammatory reactions with abundant exudation of fluid, leucocytes, and plasma protein.

Hydrolysis of protein by proteolytic enzymes brings about its ultimate disintegration into peptides and amino acids. Interruption of the process when it is well advanced results in the formation of ill-defined material long known as "peptone." It is not precipitated by heat and is dialyzable, evidently containing molecules smaller than those of the proteins from which it was derived. Peptone was found to be toxic and was the subject of much experimental study concerned with what was known as "peptone shock" or as "anaphylactoid" reactions. It is widely recognized as a useful component of media for the growth of many kinds of bacteria. Fruton and Simmonds (10) suggest that some bacteria with no proteolytic enzymes of their own may require for their maintanance nitrogen-containing compounds with small molecules that are readily diffusible.

Commercial peptone (U.S.P.) in the present experiments has proven to be a powerful inflammatory irritant when injected with sodium chloride into the peritoneal cavity in quantity as small as 0.005 cc.

Inflammation caused by trypsin or chymotrypsin evidently follows disinte-

gration of proteins by proteolytic enzymes such as that which occurs in the small intestine with digestion resulting in the liberation of amino acids.

The size and nature of the polypeptides that cause active inflammation has not been determined.

Three polypeptides, bradykinin, kallidin, and eldoisin, known to dilate blood vessels and decrease blood pressure, caused exudation of fluid in excess of that which was injected, whereas two vasopressin compounds, which increased blood pressure (see Table IX) under the conditions of these experiments caused absorption of injected peritoneal fluid. These few experiments offer tentative suggestions concerning the production of inflammatory reactions by polypeptides.

### SUMMARY AND CONCLUSIONS

The peritoneal, like the pleural cavity, gives opportunity to measure with adequate accuracy the activity of inflammatory reactions defined by movement of fluid within the cavity, by migration of leucocytes into it, and by exudation of proteins from the plasma.

The activity of inflammatory reactions caused by saccharides or by alcohols that were tested varied in accord with their molecular weight, the osmotic pressure maintained by solutions of corresponding concentration, their boiling point, or by other colligative properties.

Blood serum or globulin in the concentration with which it occurs in blood serum injected into the peritoneal cavity caused changes which differed little from those caused by physiological salt solution.

Protein with molecular weight as low as that of cytochrome C (12,000) or ovalbumin (45,000) when in dilute solution (1 per cent) were rapidly absorbed, whereas trypsin and chymotrypsin under the same conditions caused very active inflammatory reactions because they set free amino acids and perhaps polypeptides with amino acids in short chains.

The activity of inflammatory reactions caused by carbon compounds soluble in body fluids varied in accord with their colligative properties.

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